COMBINATORIAL FLUORESCENT LIBRARY BASED ON THE STYRYL SCAFFOLD

FIELD OF THE INVENTION

[0001] The present invention relates to a combinatorial library of florescent compounds based on a styryl backbone and their use as organelle-specific probes.

BACKGROUND OF THE INVENTION

[0002] Fluorescent compounds are important compounds because of their broad applications, particularly because of their highly sensitive and specific detection methods (Czarnik, 1992; Rettig et al., 1999; Slavik, 1993; Lakowica, 1999; Herman, 1998). It is desirable to obtain fluorescent compounds that fluoresce in a wide range of colors so that specific compounds can be selected for different purposes. Rational design of compounds with specific emission wavelengths and high quantum yields is difficult.

[0003] Combinatorial chemistry is a synthetic strategy that produces diverse, usually large, chemical libraries. It is the systematic and repetitive, covalent connection of a set of different monomeric building blocks of varying structure to each other to produce an array of diverse molecules. It also encompasses other chemical modifications, such as cyclizations, eliminations, cleavages, etc., that are carried

out in a manner that generates permutations and thus collections of diverse molecules.

[0004] Chemical combinatorial libraries are diverse collections of molecular compounds. These compounds are formed using a multi-step synthetic route wherein a series of different chemical modules can be inserted at any particular step in the route. By performing the synthetic route multiple times in parallel, each possible permutation of the chemical modules can be constructed. The result is the rapid synthesis of hundreds, thousands, or even millions of different structures within a chemical class.

[0005] Combinatorial synthetic and screening techniques can identify lead structures from a variety of library compounds, enhancing the success rate in developing useful new compounds while saving much time in trial and error. Following its application in drug discovery, the combinatorial approach now competes with rational design methods in the field of materials science.

[0006] A combinatorial approach has been used in developing fluorescent libraries (Seidel et al., 2001; Zhu et al., 2002; Lavastre et al., 2002). However, the spectral properties and potential applications of the reported combinatorial fluorescent libraries are still limited.

SUMMARY OF THE INVENTION

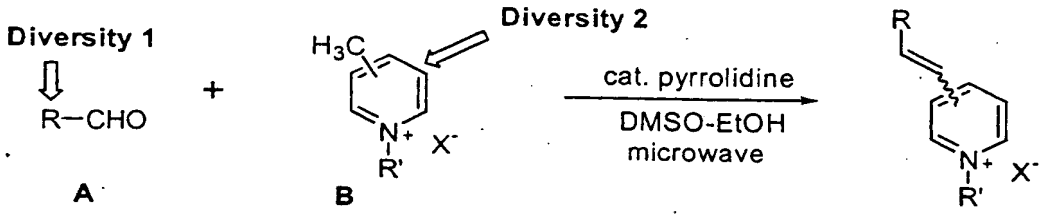
[0007] It is an object of the present invention to overcome deficiencies in the prior art.

[0008] It is another object of the present invention to produce a library of fluorescent compounds.

[0009] It is a further object of the present invention to produce a library of organelle-specific probes.

[0010] According to the present invention, a fluorescent library based upon the styryl scaffold is synthesized by condensing an aldehyde with a 2- or 4-methyl pyridinium salt as follows:

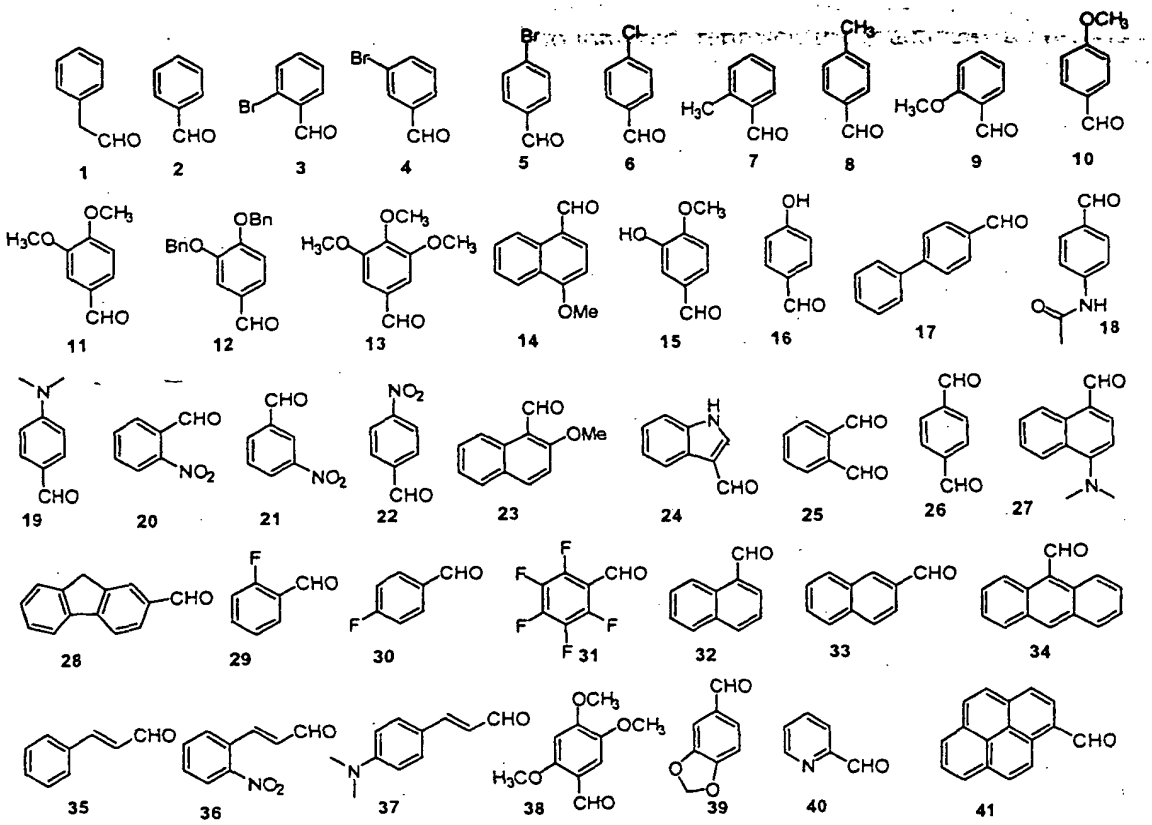
Scheme 1. Synthesis of styryl dyes



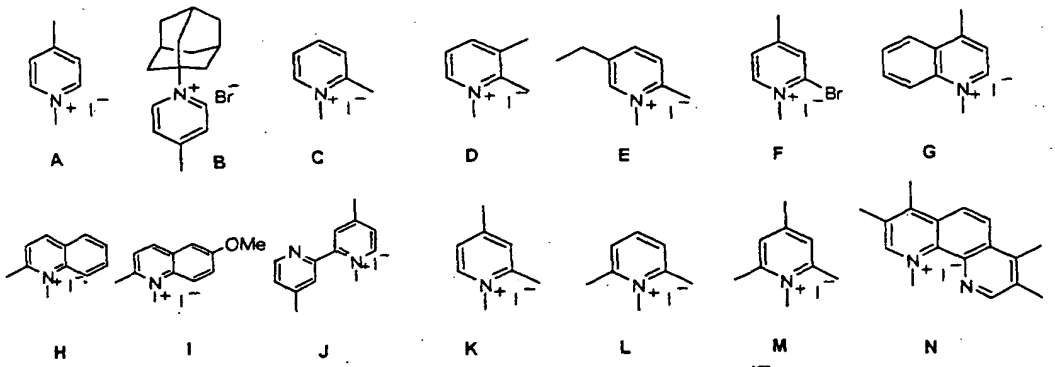
wherein R and R^1 are each selected from the group consisting of substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, alkaryl, hetereocyclic, cyclic, and fused aryl compounds, where only one methyl group is on either the 2 or 4-position.

[0011] Among the building blocks that can be used for preparing the libraries of the present invention are the following:

Building blocks A



Building blocks B



[0012] It can readily be seen that the styryl dye library of the present invention covers a broad range of colors, ranging from blue to long red, representing practically all visible colors. This broad range of colors is attributed of the structural diversity of the dyes.

[0013] It is important to note that further purification of the dyes is not required for primary analysis, as the fluorescent properties of the products are easily distinguishable from those of left-over building blocks A and B (weak fluorescence or much shorter λ_{ex} and λ_{em}). The various dyes can readily be screened to determine which dyes are best suited for detecting a specific organelle.

[0014] The synthesis of the present invention is such that the reaction mixture can be used directly in biological screening. Toxic catalysts, such as strong acids, strong bases, or toxic metals, are not present in the reaction mixture, and most of the low boiling point solvents and catalyst (e.g., pyrrolidine) were removed during microwave reaction, leaving only DMSO, a common solvent for biological sample preparation.

[0015] The synthetic compounds selected from the cell screening method exhibit a strong fluorescence increase with the addition of DNA or RNA. The fluorescence compounds will be used as sensing molecules.

BREIF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1 shows fluorescent images of representative localizations.

[0017] Figure 1A is nucleolar.

[0018] Figure 1B is nuclear.

[0019] Figure 1C is mitochondrial.

[0020] Figure 1D is cytosolic.

[0021] Figure 1E is vesicular.

[0022] Figure 1F is granular.

[0023] Figure 1G is reticular.

[0024] Figure 1H is multi-labeled.

[0025] Figure 2 shows eight selected compounds and their related derivatives.

[0026] Figure 3 shows fluorometric titration of compound 1 in a solution.

[0027] Figures 4A-4C show the absorption and fluorescence spectrum of compounds and dyes.

[0028] Figure 5A-5C show nuclear straining of compounds 1, 2, and 3, respectively.

DETAILED DESCRIPTION OF THE INVENTION

[0029] As used herein, alkyl, alkenyl and alkynyl carbon chains , if not specified, contain from 1 to 20 carbon atoms, preferably from 1 to 16 carbon atoms, and are straight or

branched. Alkenyl carbon chains of from 1 to 20 carbon atoms preferably contain 1 to 8 double bonds; the alkenyl carbon chains of 1 to 16 carbon atoms preferably contain from 1 to 5 double bonds.

[0030] Alkynyl carbon chains of from 1 to 20 carbon atoms preferably contain 1 to 8 triple bonds, and the alkynyl carbon chains of 1 to 16 carbon atoms preferably contain 1 to 5 triple bonds. The alkyl, alkenyl, and alkynyl groups may be optionally substituted, with one or more groups, preferably alkyl group substituents that may be the same or different. As used herein, lower alkyl, lower alkenyl, and lower alkynyl refer to carbon chains having fewer than or equal to about 6 carbon atoms.

[0031] As used herein an alkyl group substituent includes halos, haloalkyl, preferably halo lower alkyl, aryl, hydroxy, alkoxy, aryloxy, alkoxy, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxy, alkoxycarbonyl, oxo, and cycloalkyl.

[0032] For the present invention, "cyclic" refers to cyclic groups preferably containing from 3 to 19 carbon atoms, preferably 3 to 10 members, more preferably 5 to 7 members. Cyclic groups include hetero atoms, and may include bridged rings, fused rings, either heterocyclic, cyclic, or aryl rings.

[0033] The term "aryl" herein refers to aromatic cyclic compounds having up to 10 atoms, including carbon atoms, oxygen atoms, sulfur atoms, selenium atoms, etc. Aryl groups include, but are not limited to, groups such as phenyl, substituted phenyl, naphthyl, substituted naphthyl, in which the substituent is preferably lower alkyl, halogen, or lower alkyl. "Aryl" may also refer to fused rings systems having aromatic unsaturation. The fused ring systems can contain up to about 7 rings.

[0034] An "aryl group substituent" as used herein includes alkyl, cycloalkyl, cycloaryl, aryl, heteroaryl, optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, haloalkyl, and alkyl, arylalkyl, heteroarylalkyl, alkenyl containing 1 to 2 double bonds, alkynyl containing 1 to 2 triple bonds, halo, hydroxy, polyhaloalkyl, preferably trifluoromethyl, formyl, alkylcarbonyl, arylcarbonyl, optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, haloalkyl, alkyl, heteroarylcarbonyl, carboxyl, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, arylalkylaminocarbonyl, alkoxy, aryloxy, perfluoroalkoxy, alkenyloxy, alkynyloxy, arylalkoxy, aminoalkyl,

alkylaminoalkyl, dialkylaminoalkyl, arylaminoalkyl, amino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkylcarbonylamino, arylcarbonylamino, amido, nitro, mercapto, alkylthio, arylthio, perfluoroalkylthio, thiocyano, isothiocyano, alkylsufinyl, alkylsulfonyl, arylsulfinyl, arylsulfonyl, aminosulfonyl, alkylaminosulfinyl, dialkylaminosulfonyl, and arylaminosulfonyl.

[0035] The term "arylalkyl" as used herein refers to an alkyl group which is substituted with one or more aryl groups. Examples of arylalkyl groups include benzyl, 9-fluorenylmethyl, naphthylmethyl, diphenylmethyl, and triphenylmethyl.

[0036] "Cycloalkyl" as used herein refers to a saturated mono- or multicyclic ring system, preferably of 3 to 10 carbon atoms, more preferably from 3 to 6 carbon atoms. Cycloalkenyl and cycloalkynyl refer to mono- or multicyclic ring systems that respectively include at least one double bond and at least one triple bond. Cycloalkenyl and cycloalkynyl groups may preferably contain 3 to 10 carbon atoms, with cycloalkenyl groups more preferably containing 4 to 7 carbon atoms and cycloalkynyl groups more preferably containing 8 to 10 carbon atoms. The ring systems of the cycloalkyl, cycloalkenyl and cycloalkynyl groups may be composed of one ring or two or

more rings which may be joined together in a fused, bridged, or spiro-connected fashion, and may be optionally substituted with one or more alkyl group substituents.

[0037] The term "heteroaryl" for purposes of the present application refers to a monocyclic or multicyclic ring system, preferably about 5 to about 15 members, in which at least one atom, preferably 1 to 3 atoms, is a heteroatom, that is, an element other than carbon, including nitrogen, oxygen, or sulfur atoms. The heteroaryl may be optionally substituted with one or more, preferably 1 to 3, aryl group substituents. Exemplary heteroaryl groups include, for example, furanyl, thienyl, pyridyl, pyrrolyl, N-methylpyrrolyl, quinolyinyl and isoquinolinyl.

[0038] The term "heterocyclic" refers to a monocyclic or multicyclic ring system, preferably of 3 to 10 members, more preferably 4 to 7 members, where one or more, preferably 1 to 3, of the atoms in the ring system is a heteroatom, i.e., an atom that is other than carbon, such as nitrogen, oxygen, or sulfur. The heterocycle may be optionally substituted with one or more, preferably 1 to 3, aryl group substituents. Preferred substituents of the heterocyclic group include hydroxy, alkoxy, halo lower alkyl. The term heterocyclic may include heteroaryl. Exemplary heterocyclics include, for

example, pyrrolidinyl, piperidinyl, alkylpiperidinyl, morpholinyl, oxadiazolyl, or triazolyl.

[0039] The nomenclature alkyl, alkoxy, carbonyl, etc, is used as is generally understood by those of skilled this art. As used herein, aryl refers to saturated carbon chains that contain one or more carbon atoms; the chains may be straight or branched or include cyclic portions or may be cyclic.

[0040] The term "halogen" or "halide" includes F, Cl, Br, and I. This can include pseudohalides, which are anions that behave substantially similarly to halides. These compounds can be used in the same manner and treated in the same manner as halides. Pseudohalides include, but are not limited to, cyanide, cyanate, thiocyanate, selenocyanate, trifluoromethyl, and azide.

[0041] The term "haloalkyl" refers to a lower alkyl radical in which one or more of the hydrogen atoms are replaced by halogen, including but not limited to, chloromethyl, trifluoromethyl, 1-chloro-2-fluoroethyl, and the like. "Haloalkoxy" refers to RO- in which R is a haloalkyl group.

[0042] The term "sulfinyl" refers to -S(O)-. "sulfonyl" refers to $-S(O)_2-$.

[0043] "Aminocarbonyl" refers to $-C(O)NH_2$.

[0044] "Alkylene" refers to a straight, branched, or cyclic, preferably straight or branched, bivalent aliphatic

hydrocarbon group, preferably having from 1 to about 20 carbon atoms. The alkylene group is optionally substituted with one or more alkyl group substituents. There may be optionally inserted along the alkylene group one or more oxygen, sulfur, or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is alkyl. Exemplary alkylene groups include methylene, ethylene, propylene, cyclohexylene, methylenedioxy, and ethylenedioxy. The term "lower alkylene" refers to alkylene groups having from 1 to 6 carbon atoms. Preferred alkylene groups are lower alkylene, with alkylene of 1 to 3 atoms being particularly preferred.

[0045] The term "alkenylene" as used herein refers to a straight, branched or cyclic, preferably straight or branched, bivalent aliphatic hydrocarbon group, preferably having from about 1 to 20 carbon atoms and at least one double bond. The alkenylene group is optionally substituted with one or more alkyl group substituents. There may be optionally inserted along the alkenylene group one or more oxygen, sulfur, or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl as previously described.

[0046] As used herein, "alkynylene" refers to a straight, branched or cyclic bivalent aliphatic hydrocarbon group having from 1 to about 20 carbon atoms and at least one triple bond. The alkynylene group is optionally substituted with one or

more alkyl group substituents. There may be optionally inserted along the alkynylene group one or more oxygen, sulfur, or substituted or unsubstituted nitrogen atoms, where the nitrogen substituent is alkyl. The term "lower alkynylene" refers to alkynylene groups having from 2 to 6 carbon atoms.

[0047] The term "arylene" as used herein refers to a monocyclic or polycyclic bivalent aromatic group preferably having from 1 to 20 carbon atoms and at least one aromatic ring. The arylene group is optionally substituted with one or more alkyl group substituents. There may be optionally inserted around the arylene group one or more oxygen, sulfur, or substituted or unsubstitued nitrogen atoms, where the nitrogen substituent is alkyl.

[0048] "Heteroarylene" refers to a bivalent monocyclic or multicyclic ring system, preferably of about 5 to about 15 members, wherein one or more of the atoms in the ring system is a heteroatom. The heteroarylene may be optionally substituted with one or more aryl group substituents.

As used herein, "alkylidene" refers to a bivalent group, such as =CR'R", which is attached to one atom of another group, forming a double bond. "Arylalkylidene" refers to an alkylidene group in which either R' or R" is an aryl group.

[0049] As used herein, when any particular group, such as phenyl or pyridyl, is specified, this means that the group is substituted or unsubstituted. Preferred substituents, where not specified, are halo, halo lower alkyl, and lower alkyl.

[0050] The term "library" refers to a collection of diverse compounds, in the present case, based upon a styryl scaffold.

[0051] According to the present invention, an aldehyde is reacted with a 2- or 4-methylpyridinium salt in the presence of a secondary amine catalyst in a solvent such as a mixture of DMSO-ethanol. The secondary amine catalysts are exemplified by pyrrolidine or piperidine. However, any secondary amine can be used as a catalyst.

[0052] The reaction can be conducted in any suitable solvent, including, but not limited to, DMXO, DMF, dioxane, water, ethanol, methanol, ethyl acetate, and the like. Exogenous heat energy, such as microwave energy, is applied to the system for about 1 to about 60 minutes to form styryl-based fluorescent dyes other types of energy which can be used to heat the system can be used, such as infrared energy, a heat source, or the like.

[0053] Table I shows the fluorescence and organelle targeting data for compounds selected from the library.

Table 1. The fluorescence and organelle targeting data for the compounds selected from the library

COMPOUND LABEL	EX/EM PEAK NO.	EX(nm)	EM(nm)	LOCALIZATION NO.	LOCALIZATION
A1	1	390	490	1	CYTO
A5	1	375	540		
A12	1	330-460	540	1	MITO
A13	1	390	550		
A14	1	430(broad)	550	1	MITO
A15	1	390,420	510		
A16	1	390-420	510		
A18	1	420	610		
A19	1	460	600	1	MITO
A19				2	NUCLEOLI
A22	1	400	540		
A23	1	450 (broad)	540	1	CYTO
A23				2	MITO
A24	1	400)	530	1	CYTO
A27	1	450	640	1	CYTO
A29	1	400-420	560		
A30	1	420-440	590		
A32	1	400	510	1	MITO
A32				2	CYTO
A32				3	VESICLE
A33	1	360-420	600		
A36	1	430	700		
A37	1	460-490	580		
A38	1	410	540		
A39	1	430	540		
B1	1	360-380	480	1	CYTO
B5	1	385	570		
B9	1	390	500		
B11	1	340-440	540	1	MITO
B12	1	340-444	530	1	ER
B14	1	360-450	550	1	ER
B15	1	390,420	530		
B16	1	400	590	1	MITO
B18	1	420	580		
B19	1	380-540	610	1	MITO
B19				2	ER
B21	1	390	540		
B22	1	410-420	600	1	MITO
B23	1	380-480	530	1	CYTO
B24	1	440	530	1	MITO
B25	1	430	570	1	CYTO
B26	1	420	540		
B27	1	450(broad)	630	1	MITO
B27				2	ER
B29	1	400-420	560		
B30	1	430,450	590		
B31	1	430	580	1	MITO
B32	1	400	510	1	MITO
B33	1	350-420	500	1	MITO
B33	2	360-400	580	2	CYTO
B33				3	VESICLE
B34	1	460	610		
B36	1	420	520	1	MITO
B37	1	490,530(broad)	700	1	MITO
B38	1	400-480	580	1	NUCLEI
B38				2	MITO
B39	1	360-440	540	1	MITO
C12	1	390 (broad)	520	1	MITO?
C12				2	ER?
C13	1	380	540		
C14	1	390	530		
C15	1	390	500		
C19	1	460 (broad)	580	1	MITO
C23	1	420	530	1	CYTO
C27	1	450	620		
C32	1	390	550		
C37	1	520	680		
C38	1	420	580		
C39	1	340	520		

(**Table 1** continued)

H14	1	420-520	590	1	VESICLE
H15	1	420	610-620	1	MITO
H16	1	450	630	1	NUCLEOLI
H17	1	430	650	1	VESICLE
H17	2	420	540	2	NUCLEOLI
H18	1	430	650	1	MITO
H18				2	NUCLEOLI
H19	1	490(broad)	640	1	NUCLEOLI
H20	1	420;450-530	620	1	NUCLEOLI
H21	1	420-550	630	1	MITO
H21				2	NUCLEOLI
H23	1	420-480	580	1	VESICLE
H23				2	NUCLEOLI
H24	1	400-500	560	1	CYTO
H26	1	530	650		
H27	1	500(broad)	620	1	MITO
H28	1	350-500	660	1	NUCLEI
H31	1	420	610	1	MITO
H31				2	NUCLEI
H32	1	420	660	1	MITO
H32				2	NUCLEOLI
H33	1	340-460	620	1	MITO
H33				2	NUCLEI
H33				3	CYTO
H33				4	VESICLE
H34	1	460	650		
H39	1	530	670		
H39	1	430(broad)	560	1	CYTO
H41	1	480	640		
I1	1	460	630	1	MITO
I3	1	480	640	1	MITO
I4	1	400(broad)	620	1	GRANULE
I5	1	420	650		
I10	1	440,360	520	1	CYTO
I10	2	440,360	640	2	VESICLE
I11	1	430	560		
I12	1	360,430	560	1	VESICLE
I13	1	430	580		
I14	1	460	580-590	1	VESICLE
I15	1	360	520		
I16	1	360	530/405;540/488	1	VESICLE
I16	2	360-460	610	2	NUCLEOLI
I17	1	360,430	510	1	VESICLE
I18	1	430(broad)	650	1	NUCLEOLI
I19	1	390;400-550	630	1	NUCLEOLI
I20	1	420(broad)	620	1	NUCLEOLI
I21	1	390	620	1	VESICLE
I21				2	NUCLEOLI
I22	1	360	510		
I23	1	340-360	550		
I24	1	360	530		
I25	1	430	520		
I26	1	360-420	630		
I27	1	420	630-660	1	NUCLEOLI
I28	1	450(broad)	660	1	NUCLEOLI
I29	1	360, 420	580		
I30	1	330,430	630	1	MITO
I31	1	380	610	1	MITO
I31				2	NUCLEI
I31				3	CYTO
I32	1	360-440	610	1	MITO
I32				2	NUCLEI
I32				3	NUCLEOLI
I33	1	420	640	1	VESICLE
I33	2	320-460	560	2	MITO
I33				3	NUCLEI
I34	1	490	650		
I35	1	320-360	580	1	CYTO
I36	1	360	530		
I37	1	530	730	1	CYTO

(**Table 1** continued)

C40	1	390	610		
D23	1	420(broad)	510	1	CYTO
D37	1	470(broad)	650	1	MITO
E12	1	400	510	1	VESICLE
E12				2	ER
E13	1	380	540		
E19	1	460(broad)	580	1	MITO
E23	1	420(broad)	510	1	CYTO
E24	1	430	510		
E27	1	430	620		
E32	1	420	560		
E37	1	520	670	1	MITO
E37				2	NUCLEOLI
E38	1	430	560		
E39	1	390-420 (broad)	500		
E40	1	390	610		
F9	1	400	520		
F10	1	460	520		
F16	1	410	510		
F19	1	440(broad)	610		
F24	1	460	550	1	VESICLE
F27	1	460	640		
F32	1	410	530		
F33	1	400	510		
F38	1	460	540		
F39	1	400-420	540		
F40	1	540	640		
G7	1	440	650	1	MITO
G8	1	440	650	1	MITO
G9	1	430	630	1	MITO
G11	1	420-480	600		
G12	1	420-460	590	1	MITO
G12				2	NUCLEOLI
G13	1	420	620		
G14	1	480(broad)	620	1	MITO
G15	1	420-460	560		
G16	1	430	560		
G18	1	430	670	1	MITO
G19	1	500	670	1	MITO
G20	1	490-540	670	1	MITO
G21	1	450-550	670	1	MITO
G23	1	450-500	610	1	VESICLE
G24	1	490	610	1	MITO
G27	1	450-550(broad)	720	1	MITO
G28	1	450	620		
G29	1	450	560		
G31	1	430	650	1	MITO
G31				2	NUCLEOLI
G32	1	430	560	1	MITO
G33	1	360-470	550	1	MITO
G33				2	CYTO
G37	1	530	670		
G38	1	420	640	1	VESICLE
G38				2	CYTO
G38				3	NUCLEI
G39	1	430	590		
G41	1	500	660		
H1	1	490, 530	640	1	MITO
H2	1	480(weak)	640		
H3	1	530	640	1	MITO
H4	1	530	640		
H5	1	480	640		
H6	1	530	640		
H7	1	420	650		
H8	1	530	650		
H9	1	430 and 530	650	1	MITO
H10	1	530	650	1	MITO
H11	1	460	570		
H12	1	430	560	1	VESICLE
H13	1	420	590		

(**Table 1** continued)

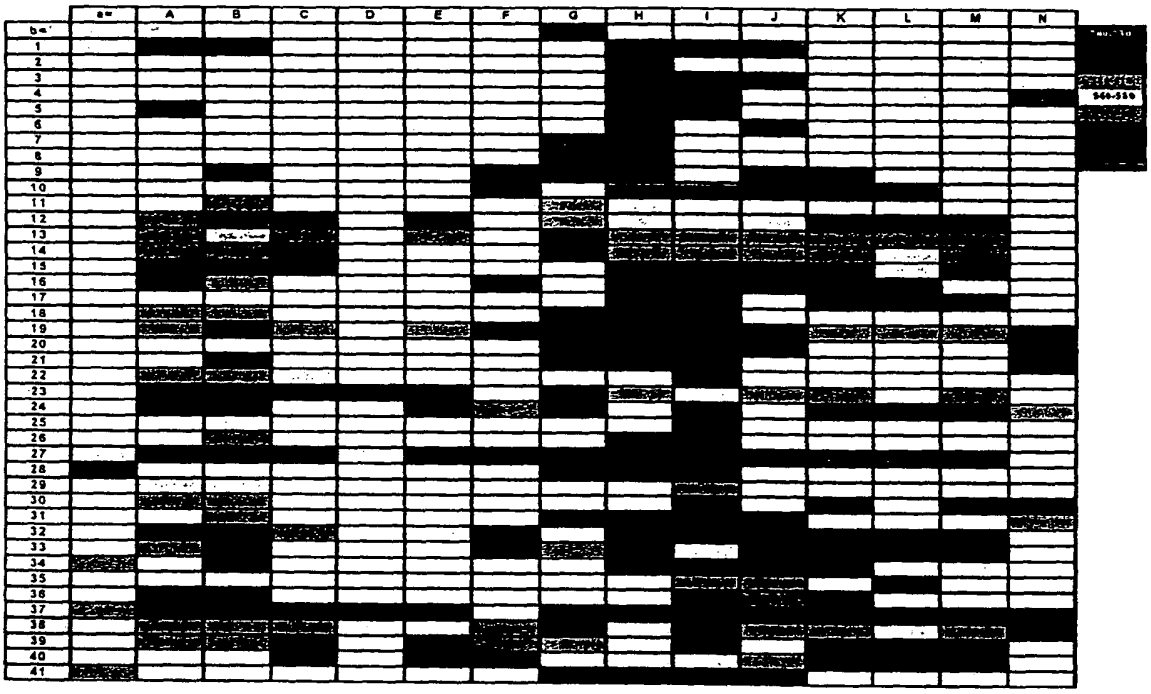
I38	1	390	620	1	CYTO
I39	1	380	500		
I41	1	480	630		
J1	1	450	620	1	MITO
J3	1	450	620	1	MITO
J6	1	400	520		
J9	1	420(broad)	520	1	MITO
J10	1	350-450	520	1	MITO
J11	1	420	560		
J12	1	350-470	560	1	VESICLE
J13	1	370,420	590		
J14	1	420-480	580		
J15	1	340-440	530	1	VESICLE
J16	1	350-460	530	1	VESICLE
J19	1	480	640	1	MITO
J20	1	420	620	1	VESICLE
J23	1	430-460	570		
J24	1	420-500	560		
J27	1	460	670		
J31	1	400,420	520	1	MITO
J32	1	350-450	530	1	MITO
J33	1	320-450	520	1	MITO
J34	1	430	630		
J35	1	340-420	580	1	CYTO
J36	1	420	540		
J37	1	550(broad)	730	1	ER
J38	1	380-500	590	1	MITO
J39	1	350-450	560	1	MITO
J40	1	400	580		
J41	1	460	630		
K9	1	400	510	1	MITO
K10	1	420	500	1	MITO
K12	1	390 (broad)	530	1	ER
K13	1	370	550		
K14	1	420	540	1	MITO
K15	1	390	510		
K16	1	400	500		
K17	1	410 (broad)	510	1	ER
K19	1	460	580	1	MITO
K23	1	460	550	1	CYTO
K24	1	380-480	520	1	MITO
K27	1	450(broad)	630	1	MITO
K30	1	410-480	610		
K32	1	320-440	510	1	MITO
K33	1	320-460	510	1	MITO
K34	1	450	610		
K36	1	410	520		
K37	1	490(broad)	670	1	VESICLE
K38	1	430 (broad)	580		
K39	1	310-440(390)	530	1	MITO
K40	1	380	610		
L10	1	420	510	1	MITO
L12	1	390	520	1	ER
L13	1	380	540		
L14	1	420 (broad)	570	1	MITO
L14				2	ER
L15	1	390	570		
L16	1	390	500		
L17	1	420	500	1	ER
L19	1	450	580	1	MITO
L23	1	420	570	1	CYTO
L24	1	430	500		
L27	1	430	620		
L32	1	400(broad)	520	1	MITO
L33	1	360-470	500	1	MITO
L35	1	420	510	1	MITO
L37	1	480	680		
L38	1	420	570		
L39	1	390	510		
L40	1	380	620		

Table 1 continued)

M12	1	400	520	1	ER
M13	1	380	540		
M14	1	420(broad)	540	1	MITO
M15	1	390	510		
M17	1	410	510	1	ER
M19	1	450	590	1	MITO
M23	1	420	540	1	CYTO
M24	1	430	520		
M27	1	440(broad)	620	1	MITO
M30	1	430	600		
M32	1	390(broad)	510	1	MITO
M33	1	320-440	500	1	MITO
M37	1	520	685		
M38	1	430	580		
M39	1	390	520	1	MITO
M40	1	460	620		
N4	1	420	610		
N19	1	580(broad)	680	1	NUCLEOLI
N20	1	580(broad)	670	1	NUCLEOLI
N21	1	420	610		
N24	1	540	590	1	CYTO
N30	1	550	590-700		
N31	1	380	600		
N37	1	470	540	1	MITO
N37	2	530,360	730	2	NUCLEOLI
N38	1	490	620		
27	1	430	570	1	GRANULE
34	1	450	550	1	GRANULE

[0054] Table 2 shows the emission colors of the fluorescent compounds from the components from the styryl dye library of the present invention. Column a shows the components in building block A, while column b shows the components in building block B.

Table 2. The emission colors of the fluorescent compounds from the Styryl dye library (a: the components in building block A; b: the components in building block B).



[0055] The compounds of the present invention can be used for organelle detection without further purification.

[0056] To obtain the results shown in Figure 1, the library compounds were incubated with live UACC-62 human melanoma cells growing on glass bottom 96-well plates, and the localizations of the different compounds in the cells were determined using an inverted fluorescence microscope (λ_{ex} = 405, 490, and 570 nm; λ_{em}>510 nm) at 1000X magnification. It was found that 119 out of 270 fluorescent compounds bind to specific organelles, such as mitochondria, ER (endoplasmic

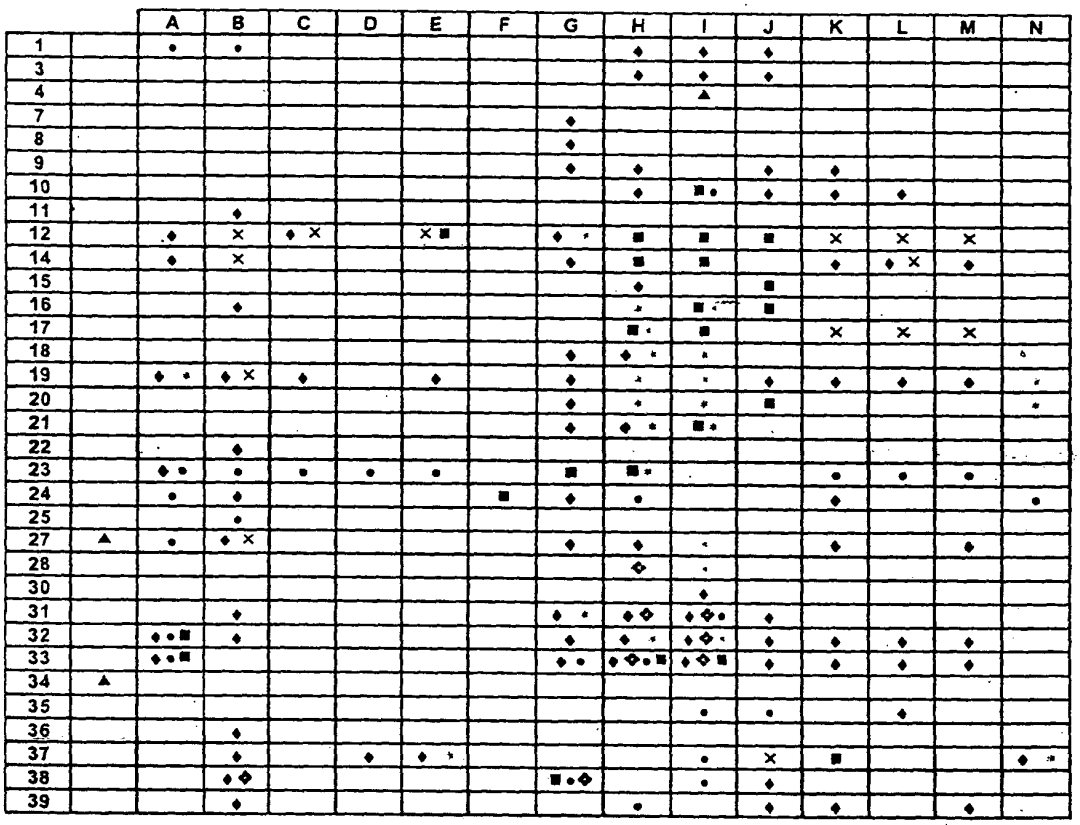
reticulum), vesicles, nucleoli, chromatin, cytoplasm, or granules.

[0057] The photographs of fluorescent images in Figure 1 show the locations of selected compounds obtained by fluorescence microscopy. Previous studies have established that there is a large voltage difference between the inside of the mitochondria and the cytosol and compounds with storing polariziability and charged compounds can interact strongly with the mitochondrial membrane. Since the library compounds are positively charged, it is not surprising that 645 out of 119 selected compounds were found to bind specifically to mitochondria.

[0058] Owing to the diversity of molecular structure, some compounds targeted organelles other than mitochondria. This encrypted interesting Structure-Localization Relationship (SLR), which can lead to rational design of molecular probes for cellular components, which opened the change for multi-color labeling using the fluorescent toolbox of the present invention.

[0059] Table 3 shows the localization distribution of the organelle specific styryl dyes of the present invention:

Table 3. The localization distribution of the organelle specific styryl dyes (❖: nucleolar; : nuclear; ♦: mitochondria; •: cytosolic; ×: endoplasmic reticular (ER); ■: vesicular; ▲: granular).



[0060] Table 4 shows the localization and color distribution of the organelle specific styryl dyes.

Table 4. The localization and color distribution of the organelle specific styryl dyes.

Color-wavelength	MITO	GRAN	VESICLE	ER	NUCLEOLI	NUCLEI	CYTO
700-730	2			1			1
	4		1		3		
	20	1	2		7		2
	9		2				3
560-580	2	1	3				5
	6	2		1		1	2
	21		3	7			5
							1
							1
	64	4	11	9	10	1	20

[0061] In UACC-62 human melanoma cell screening, only 8 out of 855 compounds showed a strong nuclear localization. The eight compounds were resynthesized in large scale for further study. The synthesis of methyl pyridium compounds was prepared by refluxing with the pyridine derivatives and iodomethan for 2 hr. Methyl pridium compound crystallized out in ethyl acetate. The condensation with aldehydes and methyl pyridium compound was performed by refluxing with piperidine for 2 hr in EtOH. After the mixture was cooled to room temperature, the crystallized compounds were filtered and washed with ethyl acetate.

[0062] With these compounds(Fig 2), we observed the fluorescence intensity change upon addition of DNA. Only compound **1** showed a strong fluorescence increase. Compound **1** is an orange solid that exhibits an excitation wavelength of λ = 413 nm and an emission wavelength of λ = 583 nm (Table 5). A linear fluorescence response was observed in the 0.05 - 100 μM range (in PBS: phosphate-buffered saline) without self-quenching or shifts in emission or excitation wavelengths. With a series of concentrations of dsDNA (double stranded DNA) added to compound **1**, a linear increase in the fluorescence intensities was observed (Fig. 3). At the highest concentration of DNA tested (50 μg/mL), the increase in fluorescence emission reached up to 13.3 times higher than

that of the free compound (Fig. 4). A blue shift of 17 nm in the emission wavelength upon DNA addition was observed, without a significant excitation wavelength shift. The structure of compound **1** includes a 2,4,5-trimethoxy group from the benzaldehyde moiety and a unique adamantyl pyridinium functionality.

[0063] Different trimethoxy isomers, **2** (3,4,5-trimethoxy) and **3** (2,3,4-trimethoxy) were synthesized to compare the positional effects of the methoxy groups in compound **1** (Fig. 2). While the responses of compound **2** and **3** to DNA treatment were simliar to that of compound **1**, the fluorescence emission increase was much smaller in compound **2** (4.3 fold) and compound **3** (1.5 fold). It is noteworthy that the intrinsic fluorescence intensity of compounds **2** or **3** is higher than that of compound **1**, but DNA treated samples showed comparable quantum yields (Table 5).

[0064] Compound **4** was also resynthesized and tested to study the structural importance of the adamantyl group in compound **1**.

[0065] Interestingly, the simple exchange of the adamantyl with a methyl group significantly reduced the DNA response in compound **4**. Therefore, it appears that both 2,4,5-trimethoxy groups and the adamantyl group are important in the specific interaction of compound **1** and DNA.

[0066] The three related compounds **1**, **2**, and **3** were incubated in live UACC-62 human melanoma cells to compare the nuclear localization properties (Fig. 5). In comparison to compound **1** in the same concentration, compounds **2** and **3** showed stronger fluorescence backgrounds and spread throughout the cytoplasm. However, compound **1** clearly shows more selective staining of the nucleus of live cells.

TABLE 5

[0067] Spectrophotometric properties of the styryl dyes

Dye	λ_{max} (nm)	λ_{em}^{free} (nm)	λ_{em}^{DNA} (nm)	ϕ_F^{free}	ϕ_F^{DNA}	$\phi_F^{DNA}/\phi_F^{free}$
Compound **1**	413	583	566	0.00024	0.0032	13.3
Compound **2**	366	553	520	0.0051	0.022	4.3
Compound **3**	370	491	502	0.0024	0.0037	1.5

[0068] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and, therefore, such adaptions and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation.

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